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List of Publications

11/27/2003

Schoumans J, Anderlid, BM, Blennow E, Teh BT, Nordenskjöld M The performance of CGH array for the detection of cryptic constitutional chromosome imbalances. Journal of Medical Genetics 2004 Jan.

Gunn Shelly, Mohammed MS, Reveles Xavier, Viskochil David, Palumbos Janice, Johnson-Pais Teresa, Hale Daniel, Lancaster Jack, Hardies Jean, Boespflug-Tanguy Odile, Cody Janine, Leach Robin Molecular Charactrization of a Patient With Central Nervous System Dysmelination and Cryptic Unbalanced translocation Between Chromosomes 4q and 18q. *American Journal of Medical Genetics* 120A:127-135 (2003)

Ulger C., Toruner GA., Alkan M., Mohammed M., Damani S., Kang J., Galante A., Soteropoulos P., Tolias P., Schawalb MN., Dermody J. Comprehensive genome-wide comparison of DNA and RNA level scan using microarray technology for identification of candidate cancer – related genes in the HL-60 cell line. Cancer Genetics and Cytogenetics 146 (2003) 1-8

G. Tachdjian, M Benkhalifa, A Aboura, I Creveaux, L. Foix-Helias, J.F. Gadisseux, O. Boespflug-Tanguy, M. Mohammed, P. Labrune De novo interstitial direct duplication of Xq21.3q25 analysed by conventional and array-based comparative genomic hybridization associated with skewed X-inactivation pattern. Submitted to Journal of Medical Genetics (2003)

J-M LaPierre, D. Sanlaville, M. Benkhalifa, M. Prieur, L Colleaux, A. Munnich, C. Turleau, M. Vekemans, S.P. Romana A Preliminary Study to assess the value of comparative genomic hybridization on a microarray to detect subtle constitutional chromosome imbalances observed in a clinical set up. Submitted to the Journal of Human Genetics (2003)

Al Mullah F, Hamadi E, Mucci M, Benkhalifa M. Genome disorder on colon cancer-An application of Microarray CGH. (2003)
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Shah S, Gregg JP, Mohammed M, Yu W, Damani S, Locker RC "BAC Microarrays". *Microarray Image Analysis – Nuts & Bolts* DNA Press, 2002.

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C. Lee, D. Drandi, P. Dal Cin, J. Gribben. Prognostic markers in CLL by array-based comparative genomic hybridization. (Abstract – American Society of Human Genetics 2003)

R.P. Ketterling, B.M. Shearer, R.C. Locker, N. Dzidic, M. bin Khalifa, M.S. Mohammed. Assessment of the Clinical Readiness of Array-CGH: A Perspective Report. (Abstract – American Society of Human Genetics 2003)

W. Yu, C. Shaw, C. Kashork, M. Santini, C. Chinault, S. Cheung, A. Beaudet. Development and validation of a pilot comparative genomic hybridization microarray for the application of clinical diagnosis. (Abstract – American Society of Human Genetics 2003)

A. Patel, W. Yu, C.A. Shaw, L. Cooper, M. Patel, M. Fishman, C. Bacino, S.W. Cheung, A. Beaudet. Identification of additional cryptic rearrangements in cytogenetically abnormal patients by a CGH microarray chip designed for clinical diagnosis. (Abstract – American Society of Human Genetics 2003)

C.J. Shaw, C.A. Shaw, W. Yu, P. Stankiewicz, L.D. White, A.L. Beaudet, J.R. Lupski. Comparative genomic hybridization using a proximal 17p BAC/PAC array detects rearrangements responsible for four genomic disorders. (Abstract – American Society of Human Genetics 2003)

J.S Hebrick, E. Kanematsu, L.R.. Osborne, P.N. Ray, J.M. Rommens, A.D. Peterson., D.E. Bulman, S.W. Scherer. Development of genomic resources and cytogenetic databases for patient sample characterization and disease gene discovery. (Abstract – American Society of Human Genetics 2003)

Benkhalifa M, Mucci M, Tachdjian G, Mohammed M, Genome profiling of blocked morulaes: Technical validation using BAC Microarray. (Abstract – International PGD Meeting 2003)

Hutto WS, Akpabio N, Zadeh SR, Chow CW, Ton T, Locker RC, Dzidic N, Xie W, Kim JW, Cai WW, Mohammed SM, Kang JJ. Covalent modification of DNA to generate oligo expression and BAC CGH microarray. (Abstract - IBC Chips to Hits 2002)

Lapierre J, Sanlaville D, Kang J, Gosset P, Ozilou C, LeLorch M, Turleau C, Mohammed M, Romania S, Vekemans M. A preliminary study to assess the predictive of a low-resolution genomic microarray in detecting chromosomal imbalances and DNA copy number polymorphisms. (Abstract – American Society of Human Genetics 2002)

Naeem R, Blackburn AC, Mohammaed MS, Naber SP, Jerry DJ. Mechanisms of tumorigenesis in mammary tumors from BALB/c-Trp53-heterozygous mice: A model for Li-Fraumeni syndrome. (Abstract – American Society of Human Genetics 2002)

Mohammed MS, Kang J, Dzidic N, Locker R, Vilain E, Bacino C, Cai WW, Naeem R. Preliminary validation of genomic microarrays for routine use in a prenatal screening for chromosomal imbalances. (Abstract – American Society of Human Genetics 2002)

Abstracts

Prognostic markers in CLL by array-based comparative genomic hybridization. *C. Lee[1,2], D. Drandi[2,3], P. Dal Cin[1,2], J. Gribben[2,3].* 1) Pathology, Brigham & Woman's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Dana Farber Cancer Institute, Boston, MA.

In Chronic Lymphocytic leukemia (CLL), genomic aberrations are important independent predictors of disease progression and survival. The most common aberrations in CLL included gains of chromosomal material in chromosome 12 as well as losses in 13q, 11q, and 17p. Fluorescence in situ hybridization (FISH) has greatly improved the ability to detect known chromosomal aberrations. Cytogenetic and molecular findings provide important diagnostic, clinical, and prognostic information, which are increasingly being used to contribute to treatment decisions and follow-up of CLL patients.

Array-based comparative genomic hybridization (array-CGH) is a molecular cytogenetic method that provides a comprehensive and genome-wide view of chromosomal imbalances. Through this technology it is now possible to identify and measure DNA sequence copy number gains and losses to identify new genetic signatures that may predict survival and to improve our knowledge of the molecular pathogenesis of this disease.

We performed array-CGH analysis on 20 patients with CLL, all of whom had their disease previously characterized by FISH in the Clinical Cytogenetics Laboratory at Brigham and Women's Hospital. Array-CGH detected the cytogenetic abnormalities previously characterized by FISH, and detected a number of cryptic abnormalities, some of which were shared among patients. Correlating the array CGH findings with patient outcome data, we have identified at least one novel cryptic deletion which appears to have a high correlation with patient prognosis.

Presented ASHG 2003

Assessment of the Clinical Readiness of Array-CGH: A Perspective Report. *R.P. Ketterling[1], B.M. Shearer[1], R.C. Locker[2], N. Dzidic[2], M. bin Khalifa[3], M.S. Mohammed[2].* 1) Dept Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 2) Spectral Genomics, Inc., Houston, TX; 3) Genechip Ltd., London, United Kingdom.

Few new technologies in clinical cytogenetics have generated such excitement as that of microarray-based comparative genomic hybridization (array-CGH). In part, the lures of this approach lay in its amenability to automation, reduced sample turn around times and independence of metaphase preparation and resolution. However, in the absence of the pictorial interpretability of a karyotype, the DNA copy number ratio profiles generated by array-CGH may preclude the correct identification and interpretation of certain chromosomal rearrangements, in particular, mosaic chromosome gains and losses and complex chromosome translocations. The objectives of the current study were to elucidate practical applications for array-CGH technology versus traditional cytogenetic methodologies, and to establish guidelines for clinical adoption. Our conclusions and insights were drawn from a large consortium of array-CGH data collected over the last two years. The results confirm a high concordance (>95%) of array-CGH data with examples of simple microdeletions/duplications, monosomies and trisomies. Mosaicism for autosomal chromosomes was consistently identified at levels of 20-25%, while this sensitivity was diminished for the sex chromosomes. Array-CGH data was informative in some samples where routine G-banding was uninformative or in retrospect, erroneously interpreted. In contrast, a few samples demonstrated the correct DNA copy number by array-CGH but incorrect karyotypic context. Additionally, 20% of samples had apparently benign, repeatable single clone aberrations, which likely represent genomic variants/polymorphisms. While the collective results of this study clearly substantiate the potential of array-CGH, it also illustrates the need for standardized data presentation and interpretation and the judicious incorporation of array-CGH into the repertoire of genetic diagnostic tools.

Presented ASHG 2003

Development and validation of a pilot comparative genomic hybridization microarray for the application of clinical diagnosis. *W. Yu, C. Shaw, C. Kashork, M. Santini, C. Chinault, S. Cheung, A. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

DNA microarray-based comparative genomic hybridization (array CGH) is a powerful technology to identify chromosome aberrations throughout the entire genome. This is accomplished by co-hybridizing differentially labeled test and reference DNAs to a microarray of genomic clones. We have previously developed BAC DNA-based arrays for this purpose including a human 1p36 microarray spanning the most distal 10.5 Mb of chromosome 1 and human 17p arrays spanning the Charcot-Marie-Tooth disease type 1A and Smith-Magenis syndrome regions. These have been used to detect deletion, duplication and triplication in samples with previously well characterized chromosome copy number change by fluorescence in situ hybridization (FISH). Here we report the development and validation of a pilot array to explore the potential applications for clinical diagnoses. The array contains a total of 72 clones including 41 subtelomeric clones from all human chromosomes and 31 clones from genomic positions corresponding to 21 different genetic disorders. An optimized experiment protocol was established and array CGH was performed to examine 11 blood samples from patients with different genetic disorders in a blinded study. The array CGH data resulted in detection of all known chromosome aberrations revealed by FISH analysis as well as some additional chromosome imbalances that were not detected with the current standard practice. As expected, the main limitation of the array CGH was found to be its inability to detect chromosome changes such as balanced translocations or inversions. In summary, our data demonstrates that array CGH is an accurate, sensitive, fast approach to analyze many chromosome imbalances and it is feasible to use this approach for clinical diagnosis of genetic disorders.

Presented ASHG 2003

Identification of additional cryptic rearrangements in cytogenetically abnormal patients by a CGH microarray chip designed for clinical diagnosis. *A. Patel[1], W. Yu[1], C.A. Shaw[1], L. Cooper[1], M. Patel[1], M. Fishman[2], C. Bacino[1], S.W. Cheung[1], A. Beaudet[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics-Neurology, Texas Children Hospital, Houston, TX.

Preliminary validation of a CGH microarray chip designed for clinical diagnosis of genetic disorders that can be routinely screened by FISH analysis found additional cryptic rearrangements while confirming the original cytogenetic abnormality in 2 out of 11 patients studied thus far. Patient 8 had an inv dup(8)(p11.23p23.1) cytogenetically and was confirmed to be all chromosome 8 material by whole chromosome 8 paint, and to be deleted for the 8p telomere by FISH analysis. Array CGH analysis confirmed loss of the 8p telomere and found a gain of the 18q telomere. Subsequent FISH analysis with the 18q telomere found the inv dup 8 chromosome to have captured the 18q telomere. This is a second case where an inv dup 8 chromosome has been has been stabilized by the 18q telomere. Patient 6 had a del(13)(q33.2) karyotype and all telomere FISH analysis with the Vysis telomere probe panel found no other abnormalities. Array CGH analysis confirmed the 13q telomere deletion but also identified a 22q telomere loss. The array 22q telomere clone is distal to the Vysis 22q telomere probe and again FISH analysis confirmed the 22q telomere deletion. Our preliminary data suggests that cytogenetically abnormal karyotypes may also harbor cryptic rearrangements that could go undetected and therefore the use of array CGH together with classical cytogenetics can provide a more accurate and sensitive clinical diagnostic screen for genetic disorders.

Presented ASHG 2003

Comparative genomic hybridization using a proximal 17p BAC/PAC array detects rearrangements responsible for four genomic disorders. *C.J. Shaw[1], C.A. Shaw[1], W. Yu[1], P. Stankiewicz[1], L.D. White[1], A.L. Beaudet[1], J.R. Lupski[1,2].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX.

Proximal chromosome 17p is a region that is rich in low copy repeats (LCRs) and prone to chromosomal rearrangements. Four genomic disorders map within the interval 17p11-p12: Charcot-Marie-Tooth disease type 1A (CMT1A), hereditary neuropathy with liability to pressure palsies (HNPP), Smith-Magenis syndrome (SMS) and dup(17)(p11.2p11.2) syndrome. While 80-90% or greater of the rearrangements resulting in each disorder are recurrent, several non-recurrent deletions/duplications of varying sizes within proximal 17p also have been characterized using fluorescence in situ hybridization (FISH). Here we tested the ability of a BAC/PAC array-based comparative genomic hybridization (array-CGH) method to detect these genomic dosage differences and map breakpoints in 26 patients with recurrent and non-recurrent rearrangements. We found that array-CGH detected the dosage imbalances resulting from either deletion or duplication in all the samples examined. Furthermore, the array-CGH approach mapped 46/47 (97.9%) of the analyzed breakpoints to within one overlapping BAC/PAC clone compared to that determined independently by FISH. In addition, array-CGH was sensitive enough to readily identify a dosage gain for clones contained on a small marker chromosome 17 present in 72% of cells. Interestingly, while a handful of clones (some containing LCRs) within the array performed less well than the majority when patient to control fluorescence ratios were analyzed, several clones that contain large LCRs did not have an adverse effect on the interpretation of the array-CGH data. Our data demonstrate that array-CGH is an accurate and sensitive method for detecting genomic dosage differences and identifying rearrangement breakpoints, even in LCR-rich regions of the genome.

Presented ASHG 2003

Covalent modification of DNA to generate oligo expression and BAC CGH microarray.

Hutto, W.S., Akpabio, N., Zadeh, S.R., Chow, C.W., Ton, T., Locker, R.C., Dzidic, N., Xie, W., Kim, J.W., Cai, W.W. [1], Mohammed, S.M., and Kang, J.J.

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Microarray technology is a revolutionary tool to understand a complex biological system in a more succinct and comprehensive manner. This high-throughput platform has been instrumental in acquiring a global and cohesive view of the molecular circuitries that govern numerous cellular processes and diseases. Here, at Spectral Genomics, Inc., we have developed an innovative technology to modify biological molecules, such as oligonucleotide and bacterial artificial chromosome (BAC) DNA to generate expression and comparative genomic hybridization (CGH) microarray chips, respectively. One distinct advantage of Spectral's patented modification technology is that the probe sequence is modified instead of the substrate surface. This approach greatly reduces the non-specific background resulting from the substrate surface, allowing a much higher signal to noise ratio (S/N). Furthermore, since the probe DNA is modified first, this allows the modified DNA to attach not only to the substrate surface, but also to each other, creating an intermolecular matrix of three-dimensional structure. In turn, these multi-level structures further increase the labeled target sequences' binding capacity such that a standard over-night stationary hybridization is used to generate a most sensitive and specific result (e.g., limits of detection (LOD) ranging from a 1.2-fold to a 40-fold ratio). Combined with Spectral's bioinformatic support, we have developed 1400 (3 Mb) and 2500 (1 Mb resolution) clone human BAC arrays, utilizing microarray as an alternative platform for the CGH analyses. Using the improved hybridization protocols, DNA samples as little as 100 ng or less can be reliably and accurately analyzed on BAC arrays in less than 48 hours. A validation study was performed on samples chosen to represent a broad spectrum of chromosome abnormalities, including examples of segmental monosomies and trisomies, double segmental imbalances, complex marker chromosomes and chromosome mosaicisms. The chromosomal aberrations were detected including entire chromosome gains/losses, whole/partial chromosome arm gains/losses, small deletions, cryptic deletions, and double segmental imbalances, and concomitant trisomy. In addition, mosaicisms of monosomy or trisomy of even the X chromosome were readily detected. Since this technology is in principle not limited to a specific number of BAC clones but rather to on-going genome sequence mapping information, it is clear that more complete genome sequencing data and progressive innovations in DNA preparation, labeling and hybridization, will introduce genome microarrays as a rapid, reliable and cost-effective way to screen the entire genome.

Presented to 2002 Chips to Hits, Philadelphia, PA.

A preliminary study to assess the predictive value of a low-resolution genomic microarray in detecting chromosomal imbalances and DNA copy number polymorphisms. *J. LAPIERRE[1], D. SANLAVILLE[1], J. KANG[2], P. GOSSET[1], C. OZILOU[1], M. Le LORCH[1], C. TURLEAU[1], M. MOHAMMED[2], S. ROMANA[1], M. VEKEMANS[1].* 1) Departement de genetique, Hopital Necker-Enfants Malades, PARIS, FRANCE; 2) Spectral Genomics Inc., Houston, TX.

Microarray-based comparative genomic hybridization (array-CGH) has been successfully used in the detection of both acquired and constitutional chromosome imbalances. Since these arrays comprise of discrete large-insert genomic clones such as BACs, they may also be used to detect DNA copy-number polymorphisms across the entire genome, which may otherwise be undetectable by conventional cytogenetic methodologies. We decided to test the capacity of a commercially available genomic microarray in detecting constitutional chromosome imbalances as well as its utility in detecting DNA copy-number polymorphisms across the genome. The array chosen for this study was a first generation microarray comprising of 1003 BAC and PAC clones (Spectral Genomics Inc.). DNA samples from patients with well characterized chromosome aberrations as well as from known normal references were tested in a blinded fashion. In accordance with the protocols established for use with these arrays, between 500ng-1g of Cy5 labeled test DNA was co-hybridized with Cy3 labeled reference DNA (forward reaction). Similarly, an equal amount of Cy3 labeled test DNA was independently co-hybridized with Cy5 labeled reference (reverse reaction). While aberrations for which clone coverage was available were detected, our findings suggest that although these arrays were initially designed to offer on average a 3Mb coverage of the genome, the resolution within particularly the subtelomeric regions is lower than 3Mb. The utility of the dye-reversal approach was highlighted by the analysis of one of the known normal reference samples. Importantly, DNA copy number polymorphisms for a single clone on 7q and 16q were observed in both our normal and abnormal DNA samples. Further studies using appropriate DNA clones are in progress to confirm these findings.

Presented ASHG 2002

Mechanisms of tumorigenesis in mammary tumors from BALB/c-Trp53-heterozygous mice: A model for Li-Fraumeni syndrome. *R. Naeem[1], A.C Blackburn[2], M.S Mohammed[4], S.P Naber[3], D.J Jerry[2].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Department of Veterinary & Animal Sciences, University of Mass. Amherst,MA; 3) Spectral Genomics, Houston, TX; 4) Department of Pathology Baystate Medical Center, Springfield, MA.

Breast cancer is the most common tumor type observed among women with Li-Fraumeni syndrome (LFS). Mammary tumors (MT) are also the most prevalent tumor type in BALB/c-Trp53-heterozygous female mice suggesting a genetic predisposition towards mammary tumorigenesis. The spectrum and histopathology of MT in this background is similar to the LFS patients, and therefore, presents a unique model for the study of breast cancer in LFS. The mechanisms of mammary tumorigenesis were examined in these p53-deficient mice. Loss of the wild type allele of Trp53 (LOH) was detected in the majority of mammary tumors from BALB/c-Trp53-heterozygous females (96%). LOH was also observed in 85% of the lymphomas and sarcomas arising in these mice which was similar to the rate of LOH for mammary tumors (P=0.35). To study the mechanism of LOH, normal tissues and tumors from 8 mice were karyotyped using short-term culture methods. FISH was used to further characterize the cytogenetic results. BAC CGH microarrays were applied to corroborate the cytogenetic results and to identify genomic regions undergoing frequent loss and gain. The mammary tumors contained a hypodiploid population of cells lacking one copy of chromosome 11 as well as a near-tetraploid population of cells. The BAC CGH data also indicated loss throughout chromosome 11. These results demonstrate that loss of the wild type allele of Trp53 in these mammary tumors results from missegregation of chromosomes rather than deletions. Preliminary results suggest that recombination within chromosome 11 occurs frequently and precedes loss of heterozygosity.

Presented ASHG 2002

Preliminary validation of genomic microarrays for routine use in prenatal screening for chromosomal imbalances. *M.S Mohammed[1], J. Kang[2], N. Dzidic[2], R. Locker[2], E. Vilain[3], C. Bacino[1], W.W. Cai[1], R. Naeem[1].* 1) Spectral Genomics, Inc, Houston, TX; 2) Dept. of Molecular and Human

Genetics, Baylor College of Medicine, Houston, TX; 3) Dept. of Human Genetics and Pediatrics, UCLA, CA.

Screening is defined as,The identification among apparently healthy individuals,of those who are sufficiently at risk of a specific disorder to justify a subsequent diagnostic test or procedure, or in certain circumstances, direct preventive action.(Nicholas J.Wald). FISH-methodologies such as AneuVisionTM have helped our approach to prenatal screening for five chromosome aneuploidies However; the use of locus-specific FISH probes to a limited subset of chromosomes precludes the ability to detect segmental imbalances of these chromosomes and other aneuploidies, which represent a significant portion of prenatal chromosome abnormalities. With the aim of developing a genome-wide prenatal screening for chromosomal imbalances we developed a high-density BAC/PAC genomic array. In addition, we have developed protocols with which DNA samples less than 100ng can be analyzed in less than 48 hours. A retrospective study was performed on discarded samples representing a spectrum of chromosome abnormalities, including segmental monosomies, trisomies, double segmental imbalances,complex marker chromosomes and examples of chromosome mosaicism. Test and reference genomic DNAs were differentially labeled with fluorochromes on Day 1 and hybridization was performed overnight. On Day 2, the BAC arrays were scanned and bioinformatically analyzed. Analysis of all samples was performed in a blinded fashion. Cyto,FISH and BAC CGH data from unbalanced aberrations for which clone coverage was available on the array showed concordance. Data from cytogenetically characterized mosaicism including X chromosome showed consistent ratio divergence. Since this technology is in principle not limited to number of BAC clones but rather to genome sequence mapping data,it is clear that more complete sequencing data and progressive innovations in DNA preparation,labeling and hybridization,will introduce genome microarrays as a rapid,reliable and comprehensive prenatal screen for all chromosomal aneuploidies and segmental imbalances.

Presented ASHG 2002

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